Determination of sea turtle intra-RBC constituents and the optimal storage media for sea turtle RBCs as part of a pre-transfusion protocol

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Introduction

There are seven sea turtle species in the world, and five of these species are found in Florida, including the threatened loggerhead (Caretta caretta) and endangered green sea turtle (Chelonia mydas). These species often suffer from fibropapilloma1, an aggressive virus that is commonly seen as cauliflower shaped masses on the flippers, neck, and eyelids2, and surgery is often the only option for treatment. Treatment can often be necessary in sea turtles undergoing tumor removal surgery following the diagnosis of fibropapillomatosis. Due to their size difference, it is thought that the larger loggerheads might prove to be optimal blood donors for the smaller greens. However, current recommendations for transfusions and red blood cell (RBC) storage of sea turtle RBCs are based on studies in mammals and a single freshwater turtle study3. Evidence-based guidelines and protocols for blood donations and transfusions in sea turtles do not exist to date. An important part of developing a transfusion protocol in sea turtles involves determining the intracytoplasmic constituents of sea turtle RBCs and ascertaining the optimal storage solution for RBCs. To date, studies evaluating intracytoplasmic RBC constituents and the effects of various storage media on sea turtle RBCs have not been performed.

Hypothesis

We hypothesized that 1) Sea Turtle RBCs will have similar intra-cellular constituent to those determined in mammals, and 2) Sea Turtle whole blood can be adequately stored in RBC storage media commonly used to store and preserve mammalian RBCs, ACD, CPD, or CPDA-1.

Materials and Methods

**Phase 1: Blood (3 mL) was collected from the dorsal cervical vein using a 21 gauge 2 inch needle and a 3cc syringe from 5 greens and 8 loggerheads at the Loggerhead Marinelife Center or nesting female Loggerheads on Loggerhead Beach (Figure 1) in West Palm, Florida. Samples were collected into lithium heparin green top tubes and immediately packed in a cooler with ice packs collected in the field or a refrigerator prior to overnight shipment to UC Davis.**

**Phase 2:** Blood samples from the same blood donors were evaluated on Day 0 and stored for 14 days in 3 conditions: ACD, CPD, and CPDA-1. Samples were analyzed for 24-hour storage on Day 14.

**Phase 3:** Blood samples were evaluated for Day 0 whole sample hemoglobin, pH, and other hematological and biochemical parameters. In addition, a 3-mL sample was collected into a heparinized tube and centrifuged to generate a clear supernatant with a pellet of bound hemoglobin at the bottom (Figure 4) of the tube. This mixture was vortexed, rocked (Figure 3), and lysed, and analyzed via Chemistry Analyzer in order to determine their intracellular constituents.

**Phase 4:** Blood samples collected as part of Day 0 pre-surgery, were also submitted for electrolyte evaluation.

**Phase 5:** Blood samples were evaluated for Day 0 whole sample hemoglobin, pH, and other hematological and biochemical parameters. In addition, a 3-mL sample was collected into a heparinized tube and centrifuged to generate a clear supernatant with a pellet of bound hemoglobin at the bottom (Figure 4) of the tube. This mixture was vortexed, rocked (Figure 3), and lysed, and analyzed via Chemistry Analyzer in order to determine their intracellular constituents. The lysed supernatant that had not been treated with Hemoglobinst was also submitted for electrolyte evaluation.

**Phase 6:** Blood samples collected as part of Day 0 pre-surgery, were also submitted for electrolyte evaluation.

**Phase 7:** Blood samples were evaluated for Day 0 whole sample hemoglobin, pH, and other hematological and biochemical parameters. In addition, a 3-mL sample was collected into a heparinized tube and centrifuged to generate a clear supernatant with a pellet of bound hemoglobin at the bottom (Figure 4) of the tube. This mixture was vortexed, rocked (Figure 3), and lysed, and analyzed via Chemistry Analyzer in order to determine their intracellular constituents. The lysed supernatant that had not been treated with Hemoglobinst was also submitted for electrolyte evaluation.

**Phase 8:** Blood samples collected as part of Day 0 pre-surgery, were also submitted for electrolyte evaluation.

**Figure 1:** Female loggerhead nesting on Juno Beach, when blood collection would occur. Used with permission from Dr. Charlie Manire.

**Figure 2:** Lyzed RBCs from 4 Green Sea Turtles.

**Figure 3:** Hemoglobinst and RBC mixture at rocking stage.

**Figure 4:** Samples Post-Hemoglobinst agitation. Clear supernant remains for analysis.

**Figure 5:** Hematocrit Tube post-centrifulation, Day 0, Loggerhead 1, ACD.

**Figure 6:** Loggerhead 1 hemolysed blood, Day 0, stored in ACD, CPD, CPDA-1 (from left to right).

**Table 1:** Green Sea Turtle 1-5 (Chelonia mydas) intra-RBC constituents

<table>
<thead>
<tr>
<th>Constituent</th>
<th>ACD</th>
<th>CPD</th>
<th>CPDA-1</th>
</tr>
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<tbody>
<tr>
<td>K (mmol/l)</td>
<td>25.7</td>
<td>18.9</td>
<td>17.6</td>
</tr>
<tr>
<td>Phos (mg/dl)</td>
<td>6.7</td>
<td>8.4</td>
<td>7.6</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>4.5</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>25.0</td>
<td>19.0</td>
<td>16.0</td>
</tr>
<tr>
<td>Mg (mg/dl)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>GLDH (U/l)</td>
<td>2.5</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>5.7</td>
<td>4.5</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Significant differences were noted between ACD and CPD for phosphorus and glucose, and between CPDA-1 and ACD for phosphorus, glucose, and pH (p < 0.05).

**Discussion**

We had hypothesized that sea turtle RBCs would have similar intracellular constituents to those identified in mammalian species, and our results support our hypothesis. It is interesting to note that both Green and Loggerhead sea turtles are both high potassium RBC species. This interpretation was based upon mammalian studies, in which those species with potassium levels higher than 13 mmol/l are noted to be low potassium RBC species. Canines are considered to be a low potassium red cell species, and this is due to their lack of a sodium-potassium ATPase membrane pump in their mature RBCs. Therefore, we propose that both Green and Loggerhead sea turtles’ erythrocyte membranes maintain a sodium-potassium ATPase pump, which could also account for their low potassium and chloride RBC values. Lastly, there was no statistical significant difference between electrolyte and enzyme measurements between the two species, which supports our belief that Loggerhead sea turtles may be suitable blood donors for Green sea turtles. While we had hypothesized that three RBC storage media commonly used to store and preserve mammalian RBCs (ACD, CPD, or CPDA-1) would be adequate for use in both Green and Loggerhead sea turtle, our results suggest otherwise. Three out of four of the collected samples were almost completely hemolysed within 24 hours post collection, and the fourth sample was almost completely hemolysed by 48-hour post collection. Much of the blood samples were unable to be quantified due to marked levels of hemolysis, such as PCV (Figure 3) in which a spun hematocrit tube has no discernable border between the plasma and the RBC layer. Similarly, the deep red color of the blood within the tubes and bags and various indicators of hemolysis (Figure 6).

Furthermore, the low number of intact red blood cells, elevated AST, and acidic pH support RBC lysis and hemolysis. The American Association of Blood Banks and FDA requires a transfusion product with a 24 hour survival rate of at least 70% of transfused cells. Given our findings and routine anticoagulants used for blood storage for transfusion purposes in mammals cannot be recommended for use in Green and Loggerhead sea turtles at this time. Sea turtle blood is routinely collected in heparin bags for CABC purposes as the use of EDTA tubes is associated with hemolysis4. With this in mind, we propose that the observed hemolysis in phase 2 of this study may be secondary to sea turtle membrane ion channel interactions with commonly used RBC anticoagulant solutions. Future studies will seek to explore sea turtle membrane ion transport mechanisms.

**Conclusion**

1. Green and Loggerhead sea turtles are both high potassium RBC species
2. Routine anticoagulants used in mammals for transfusion purposes are not adequate for use with Green and Loggerhead sea turtle RBCs.

**Acknowledgements**

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**References**